II. The Rejections Under 35 U.S.C. § 112, First Paragraph, Must Be Withdrawn

At page 2 of the Office Action, the Examiner maintained the rejection of claims 89-126, under 35 U.S.C. § 112, first paragraph, as allegedly not enabled. Applicants respectfully traverse this rejection. A *prima facie* case of non-enablement has not been established.

A. The Standard for Enablement

The initial burden of proving that a specification is non-enabling is on the Examiner. Under controlling Federal Circuit precedent, it is axiomatic that a specification is presumed to be enabling unless the Examiner provides acceptable objective evidence or sound scientific reasoning showing that it would have required undue experimentation for one of ordinary skill in the art to make and use the claimed invention. In *In re Cortright*, 165 F.3d 1353, 49 USPQ2d 1464, 1469 (Fed. Cir. 1999), the court stated that the PTO cannot make a section 112, first paragraph, rejection for lack of enablement, unless the PTO "has reason to doubt the objective truth of the statements contained in the written description." *See also In re Marzocchi*, 439 F.2d 220, 223, 169 USPQ 367, 369 (CCPA 1971). Under *Cortright* and *Marzocchi*, the claims in an application are presumed to be enabled, unless proven otherwise.

Further, it is well-established that some experimentation is permitted, so long as it is not "undue." *See In re Wands*, 8 USPQ2d 1400, 1404 (Fed. Cir. 1988) ("Enablement is not precluded by the necessity for some experimentation such as routine screening. However, experimentation needed to practice the invention must not be undue experimentation.") (citations omitted); *see also Ex parte Forman*, 230 USPQ2d 546, 547 (BPAI 1986) ("The ultimate question . . . is whether or not the specification contains a sufficiently explicit

disclosure to enable one having ordinary skill in the relevant field to practice the invention claimed therein without the exercise of undue experimentation.").

Thus, the initial burden is not on Applicants to prove that a claimed invention is enabled. Instead, the initial burden is on the Examiner to establish a *prima facie* case of non-enablement, by providing objective evidence or sound scientific reasons why the claimed invention is allegedly not enabled. As discussed below, the Examiner has not provided reasons sufficient to create a *prima facie* case of non-enablement.

B. A Prima Facie Case of Non-Enablement Has Not Been Established

1. The Examiner's Proffered Reasons Are Insufficient To Establish a Prima Facie Case of Non-Enablement

As discussed below, the Examiner's proffered reasons are insufficient to establish that the claimed methods, composition, and product of manufacture would not have been enabled using embryonic stem cells other than the specific mouse embryonic stem cells exemplified in the present specification, and using any serum-free medium other than the serum-free medium exemplified in Tables 1-3 (pp. 27-29) of the present specification.

2. Given the Disclosure of the Present Specification, the Claimed Methods Could Have Been Practiced Without Undue Experimentation

a. The Claims Relate to In Vitro Expansion of Embryonic Stem Cells, Not to Germline Transmission of Genetic Information

At page 14 of the present specification, lines 1-3, Applicants define the term "embryonic stem cell" and "pluripotent embryonic stem cell" to "refer to a cell which can give rise to many differentiated cell types in an embryo or an adult, including the germ cells (sperm

and eggs)." Nevertheless, the claimed methods, composition and product of manufacture relate to expansion of embryonic stem cells in serum-free culture. Thus, the claimed methods, composition and product of manufacture relate to *in vitro* expansion of embryonic stem cells, not to the *in vivo* effect of embryonic stem cells that have been inserted into a host animal blastocyst.

In analyzing whether the claimed methods, composition and product of manufacture are enabled, the Examiner should focus on what is claimed. Here, the Examiner has misinterpreted the claims to include an *in vivo* limitation, such that for a cell to be regarded by those of ordinary skill in the art as an embryonic stem cell, the cell must exhibit transmission of genetic information *in vivo*. *See* the Office Action at 3-4. However, the claims do not recite such a limitation.

Further, for cells to have been regarded as "embryonic stem cells," it is not necessary for the cells to have been demonstrated to have been capable of exhibiting transmission. Those of ordinary skill in the art regarded cells from many species as embryonic stem cells, even before genetic transmission experiments were successful. Those of ordinary skill in the art regarded cells as embryonic stem cells by virtue of (1) morphological characteristics, (2) the ability to be maintained in culture in an undifferentiated state, and/or (3) the ability to control the differentiation of the cells in culture.

Once cells were deemed embryonic stem cells, they were tested for their ability to promote germline transmission of genetic information, exhibited by the production of chimeric animals. Even if germline transmission experiments were not successful, the cells were not automatically assumed not to be embryonic stem cells, because technological obstacles to

germline transmission may prevent the transmission of genetic information, even from embryonic stem cells.

The Examiner has not provided any evidence or technical reasons why one of ordinary skill in the art would have doubted that embryonic stem cells could have been identified based on (1) morphological characteristics, (2) the ability to be maintained in culture in an undifferentiated state, or (3) the ability to control the differentiation of the cells in culture. It is improper for the Examiner to require that before a cell could have been regarded as an embryonic stem cell, transmission of genetic information *in vivo* must have been shown. Further, none of the art cited by the Examiner (*i.e.*, Keller, Bradley, Seamark, Matsui or Baribault) cast doubt on the assumption that claims 89-126 are enabled.

b. Only Routine Experimentation Would Have Been Required to Have Used Embryonic Stem Cells Other Than Those Exemplified in the Present Specification

The Examiner has provided no evidence or sound technical reasons why the methods, composition and product of manufacture, which, *as claimed*, relate to *in vitro* expansion of embryonic stem cells, would allegedly not have been enabled with embryonic stem cells other than the mouse cells exemplified in the present specification.

Indeed, the pre-filing date literature is replete with examples that those of ordinary skill in the art have developed embryonic stem cell lines from multiple strains of mice, and from non-mouse species. Following are such examples from the literature:

i. Mouse Embryonic Stem Cells

Labosky, P.A. *et al.*, *Development 120*: 3197-3204 (1994) ("Labosky," Attachment 6 to the reply filed August 9, 2000) discloses the culture of mouse embryonic stem cells, and the production of chimeric mice using a mouse embryonic stem cells. Magin, T.M. *et al.*, *Nucleic Acids Res. 20*: 3795-3796 (1992) ("Magin," Attachment 7 to the reply filed August 9, 2000) discloses the isolation and culture of mouse embryonic stem cells, and the production of chimeric mice using mouse embryonic stem cells. Thompson, S. *et al.*, *Cell 56*: 313-321 (1989) ("Thompson," Attachment 13 to the reply filed August 9, 2000) discloses the isolation and culture of mouse embryonic stem cell lines, and the production of chimeric mice. Thus, at the filing date of the application to which the present application claims priority, mouse embryonic stem cells other than those exemplified in the present application were known to exist.

At page 7 of the Office Action, the Examiner stated "[w]ith regard to the references directed to establishment of mouse embryonic stem cells, as indicated in the above rejection, establishment of mouse embryonic stem cells is known in the art." Based on the teaching of the prior art, and the Examiner's above-quoted statement regarding mouse embryonic stem cells, it is not clear to Applicants why the Examiner maintains that mouse cell lines other than those exemplified in the present specification are allegedly not enabled.

In light of the above-cited literature, the Examiner has not provided objective evidence or sound technical reasons to doubt that, prior to the January 10, 1997 priority filing date of the present application, those of ordinary skill in the art could have cultured cells that they regarded as pluripotent, embryonic stem cells from multiple strains of mice.

ii. Embryonic Stem Cells From Species Other Than Mouse

Doetschman, T. *et al.*, *Developmental Biology 127*: 224-227 (1988) ("Doetschman," Attachment 2 to the reply filed August 9, 2000) discloses the "establishment and maintenance of *hamster ES* [embryonic stem] *cell lines and show that they are highly pluripotent*." Doetschman at 224, left column, last paragraph (emphasis added). At page 7 of the Office Action, the Examiner stated that Doetschman "indicates that experiments were being carried out to determine if the hamster ES cells can colonize the germ line when introduced into hamster blastocysts (see page 227, left column, last paragraph."). However, that statement by Doetschman fails to support a *prima facie* case of non-enablement. Even though germ-line colonization transmission had not been demonstrated for the hamster cells, the authors nonetheless regarded the cells as embryonic stem cells. Nothing in Doetschman suggests that the cells were not regarded as embryonic stem cells. Indeed, throughout the article, the cells are referred to as "ES" [embryonic stem] cells. Thus, rather than supporting the Examiner's argument for non-enablement, Doetchman supports Applicants' argument for enablement.

In the Office Action, the Examiner did not comment on Du, F. *et al.*, *J. Reprod. Fertility 104*: 219-223 (1995) ("Du," Attachment 3 to the reply filed August 9, 2000). Du discloses that "[p]luripotent embryonic stem (ES) cells or ES-like cells have been derived from preimplantation embryos of several mammals including mice . . . , hamsters . . . , pigs . . . , cattle . . . , mink . . . , rats . . . , and rabbits . . . ." Du at page 219, left column, first paragraph (citations omitted). Du also discloses the isolation and culture of putative rabbit embryonic stem cells, and that nuclear transfer of the cells leads to normal blastocyst development.

Graves, K.H. *et al.*, *Mol. Reprod. Development 36*: 424-433 (1993) ("Graves," Attachment 4 to the reply filed August 9, 2000) discloses the isolation and culture of putative embryonic stem cell lines from rabbit. At page 7 of the Office Action, the Examiner stated that Graves teaches that "it remains to be tested whether these a [sic] well as other presumptive ES cells (hamster, pig, sheep, cow, and mink), are totipotent, *i.e.*, if the cells can give rise to viable progeny when introduced into recipient blastocysts (see page 432, left column)." However, that statement by Graves fails to support a *prima facie* case of non-enablement. Even though germ-line colonization transmission had not been demonstrated for the rabbit cells, the authors nonetheless regarded the cells as embryonic stem cells. Nothing in Graves suggests that the cells were not regarded as embryonic stem cells. Indeed, Graves teaches that "[t]his report describes the first successful isolation of presumptive ES cells from the rabbit as well as preliminary characterization of their in vitro properties." *See* Graves at 428, last paragraph. Thus, Graves supports Applicants' argument for enablement.

Iannaccone, P.M. *et al.*, *Developmental Biology 163*: 288-292 (1994) ("Iannacone," Attachment 5 to the reply filed August 9, 2000) discloses the isolation and culture of rat embryonic stem cells, and the production of chimeric rats using rat embryonic stem cells. At page 7 of the Office Action, the Examiner cited Iannacone at page 291 for the statement that "as yet our [rat] chimeras have not demonstrated germ line transmission." However, that statement by Iannacone fails to support a *prima facie* case of non-enablement. Even though germ-line colonization transmission had not been demonstrated for the rat cells, the authors nonetheless regarded the cells as embryonic stem cells.

Indeed, throughout the article, the cells are referred to as "ES" [embryonic stem] cells. In addition, Iannacone states that "[p]luripotent cells have been isolated from mink . . . , pig . .

. , and hamster . . . but so far there are no published accounts of chimera formation with stem cells from species other than mouse." (Citations omitted.) Iannacone at 290, right column, last paragraph. Thus, consistent with Applicants' argument, those of ordinary skill in the art regarded embryonic stem cells as "pluripotent cells," even if germline transmission had not been demonstrated for those cells.

Notarianni, E. *et al.*, *J. Reprod. Fert., Suppl. 41*: 51-56 (1990) ("Notarianni," Attachment 8 to the reply filed August 9, 2000) discloses the isolation, maintenance, and differentiation in culture of pluripotential embryonic cell lines from pig. "We conclude that pluripotent embryonic lineages may be derived from the pig and can be maintained in culture." Notarianni at 55, last paragraph. At page 7 of the Office Action, the Examiner stated that "Notarianni *et al.* is directed to pluripotential embryonic cell lines from pig, not embryonic stem cells." This statement fails to support a *prima facie* case of non-enablement. Indeed, Notarianni's disclosure of embryonic stem cells from pig supports Applicants' argument for enablement.

In the Office Action, the Examiner did not comment on Petitte, J. N. *et al.*, U.S. Patent No. 5,340,740, issued August 23, 1994 ("Petitte," Attachment 9 to the reply filed August 9, 2000). Petitte discloses the isolation and culture of chicken embryonic stem cells.

In the Office Action, the Examiner did not comment on Saito, S. *et al.*, *Roux's Arch. Dev. Biol. 201*: 134-141 (1992) ("Saito," Attachment 10 to the reply filed August 9, 2000). Saito discloses the isolation and culture of bovine stem cell-like cell lines.

Strelchenko, N. *et al.*, *Theriogenology 41*: 304 (1994) ("Strelchenko," Attachment 11 to the reply filed August 9, 2000) discloses the isolation and culture of bovine embryonic pluripotent cell lines. At page 7 of the Office Action, the Examiner stated that "Strelchenko *et*

al. is directed to pluripotential embryonic cell lines from bovine, not embryonic stem cells." This statement fails to support a *prima facie* case of non-enablement. Indeed, Strelchenko's disclosure of embryonic stem cells from pig supports Applicants' argument for enablement.

In the Office Action, the Examiner did not comment on, Sukoyan, M.A. *et al.*, *Molec. Reprod. Develop. 33*: 418-431 (1992) ("Sukoyan," Attachment 12 to the reply filed August 9, 2000). Sukoyan discloses the isolation and culture of mink embryonic stem cell lines, and the production of chimeric mink.

Thompson, J.A. *et al.*, *Proc. Natl. Acad. Sci. USA 92*: 7844-7848 (1995) ("Thompson," Attachment 14 to the reply filed August 9, 2000) discloses the isolation and culture of a primate embryonic stem cell line. Thompson, J.A., U.S. Patent No. 5,843,780, filed January 18, 1996, issued December 1, 1998 (Attachment 15 to the reply filed August 9, 2000) discloses the isolation and culture of a primate embryonic stem cell line. In the Office Action, the Examiner did not comment on either document by Thompson.

In the Office Action, the Examiner did not comment on Wakamatsu, Y. *et al.*, *Molec. Marine Biology Biotechnology 3*: 185-191 (1994) ("Wakamatsu," Attachment 16 to the reply filed August 9, 2000). Wakamatsu discloses the establishment of a pluripotent cell line derived from a fish.

In light of the above-cited literature, the Examiner has not provided objective evidence or sound technical reasons to doubt that, prior to the January 10, 1997 priority filing date of the present application, those of ordinary skill in the art could have cultured cells that they regarded as pluripotent, embryonic stem cells from species other than mouse.

c. Only Routine Experimentation Would Have Been Required To Develop Serum-Free Cell Culture Media That Support the Expansion of Embryonic Stem Cells In Vitro

At pages 8-9 of the Office Action, the Examiner stated:

> While one of ordinary skill in the art could readily manipulate medium formulations, given the unpredictability in the art of culturing embryonic stem cells, it would require undue experimentation to formulate a medium composition which supports *in vitro* expansion of embryonic stem cells.

Applicants respectfully disagree. One of ordinary skill in the art would not have practiced the claimed invention in a vacuum. Instead, the artisan would have had the benefit of Applicants' disclosure, in which Applicants have provided one of ordinary skill in the art with starting points, from which formula ingredients and concentrations may be optimized.

For example, Applicants have provided the ingredients and concentrations provided in Tables 1-3, at pages 27-30 of the present specification. Further, in the specification at page 22, lines 3-9, Applicants disclose:

> The concentration ranges within which ingredients are believed to support the growth of ES and other cells in culture are listed in Tables 1-3. These ingredients can be combined to form the cell culture medium supplement of the present invention. As will be readily apparent to one of ordinary skill in the art, the concentration of a given ingredient can be increased or decreased beyond the range disclosed and the effect of the increased or decreased concentration can be determined using only routine experimentation.

Serum-free medium development is an empirical art, and necessarily involves trial and error. However, simply because trial and error would have been required to have made a serum-free medium for use in the claimed methods, composition, and product of manufacture, that does *not* mean that the amount of trial and error required would have been *undue*. *See*

Wands, 8 USPQ2d 1400, 1404 (Fed. Cir. 1988) ("Enablement is not precluded by the necessity for some experimentation such as routine screening.").

The art is replete with evidence that those of ordinary skill in the art could have taken Applicants' disclosed medium formulation and developed a significant number of operative formulations containing either fewer ingredients than are recited in the present specification. For example, appended, collectively, as Attachment 17 to the reply filed August 9, 2000, are pages 80-81 of Freshney, R.I., "Culture of Animal Cells: A Manual of Basic Technique, Second Edition, Alan R. Liss, Inc., New York (1987) ("Freshney"). Freshney provides:

> As with medium and serum section, trial and error may be the only method to select the correct supplements. If a group of compounds is found to be effective in reducing serum supplementation, *the active constitutents [sic] may be identified by systematic omission of single components, and then their concentrations optimized.* [Ham, 1984].

Freshney at page 81, left column, first full paragraph (emphasis added) (bracketed citation to "Ham, 1984" in original).

Thus, Freshney supports Applicants' argument that only routine experimentation would have been required to practice the claimed methods. Freshney reflects that those of ordinary skill in the art of cell culture media development have long been accustomed to identifying active ingredients in new serum-free medium formulations by systematically omitting single components, and optimizing ingredient concentrations. Thus, those of ordinary skill in the art could have taken Applicants' disclosed medium formulation and developed a significant number of operative formulations containing fewer ingredients than are recited in Tables 1-3 of the present specification.

Appended as Attachment 18 to the reply filed August 9, 2000 is copy of Jayme, D.W. and D.F. Gruber,[1] "Development of Serum-Free Media and Methods for Optimization of Nutrient Composition," in *Cell Biology: A Laboratory Handbook*, Celis, J.E., Ed., Vol. 1, Academic Press, San Diego, California (1994), pages 18-24 ("Jayme"). At page 21, first full paragraph, Jayme states, "[k]ey steps toward development of a serum-free formulation for the desired cell type include the following," and Jayme then provides a standard approach for identifying medium ingredients and concentrations.

Appended as Attachment 19 to the reply filed August 9, 2000 is a copy of Gruber, D.F. and D.W. Jayme,[2] "Cell and Tissue Culture Media: History and Terminology," in *Cell Biology: A Laboratory Handbook*, Celis, J.E., Ed., Vol. 1, Academic Press, San Diego, California (1994), pages 451-458 of the Appendices chapter ("Gruber"). Gruber teaches:

> The nutrient environment or "acceptable" cell growth is basically simple, yet specifically complex. The cellular milieu must address numerous physical and chemical factors, including temperature, osmotic pressure, hydrogen ion concentration (pH), and the presence of inorganic salts, essential (and nonessential) amino acids, vitamins, dissolved gasses, and other unidentified growth materials provided through supplementation(s). *Each of these interdependent requirements had to be assessed, independently and jointly, to ascertain optimal nutrient levels.*

Gruber at 453 (emphasis added).

Appended as Attachment 20 to the reply filed August 9, 2000 is Ham, R.G. and W.L. McKeehan, *Methods In Enzymology LVIII*: 44-93 (1979) ("Ham"). At pages 75-77, Ham

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[1] The authors are employees of Life Technologies, Inc., the original assignee of the present application. Invitrogen Corporation became the owner of the above-captioned application by virtue of a merger on September 14, 2000, with Life Technologies, Inc.

[2] The authors are employees of Life Technologies, Inc., the original assignee of the present application.

describes a cell culture assay for use in determining which ingredients in a medium are required for cell growth. Ham reflects that the ability to systematically study individual ingredients was a skill possessed by those of ordinary skill in the art.

> *Systematic study* of the effects of individual variables requires specifically that all interactions be recognized and that all other significant variables be held constant *while the effects of each single variable or interacting group on cellular multiplication* are being studied.

Ham at 79, last full paragraph (emphases added). At page 92, Ham provides a generalized sequence of steps for minimizing serum requirements and for developing defined media.

In light of the above-cited literature, the Examiner has not provided objective evidence or sound technical reasons to doubt that those of ordinary skill in the art could have taken Applicants' disclosed medium formulation and developed a serum-free medium containing fewer ingredients than are recited in Tables 1-3 of the present specification.

3. Summary

In summary, the reasons proffered by the Examiner fail to establish a *prima facie* case of non-enablement, because neither objective evidence nor sound scientific reasoning has been presented to show that the claimed invention is not enabled. In view of Applicants' application and knowledge possessed by one of ordinary skill in the art, the claimed invention is enabled. Given Applicants' disclosure, including the examples and figures in the present application, and the knowledge possessed by those of ordinary skill in the art when Applicants' application was filed, the claimed invention could have been practiced without undue experimentation.

Applicants respectfully request that this rejection be reconsidered and withdrawn.

C. With Respect to the Rejection of Claims 117-121 in the Office Action Mailed March 10, 2000, a Prima Facie Case of Non-Enablement Has Not Been Established

In the Office action mailed March 10, 2000, the Examiner alleged that claims 117-121 are not enabled, because the medium disclosed in the present specification allegedly could not have been used to differentiate embryonic stem cells under serum-free conditions without undue experimentation. *See* the Office action mailed March 10, 2000 at page 7.

Claims 117 and 118 are directed to a method of causing embryonic stem cells to differentiate into a particular type of cell in serum-free culture. Claim 119 depends from claim 117 or 118, and recites that the method further comprises seeding embryonic stem cells upon a layer of feeder cells. Claim 120 depends from claim 117 or 118, and recites that one or more growth factors that prevents differentiation of embryonic stem cells is added to the culture medium. Claim 121 depends from claim 117 or 118, and recites that one or more growth factors that facilitates differentiation of embryonic stem cells is added to the culture medium.

At pages 20-22 of the reply filed August 9, 2000, Applicants argued why a *prima facie* case of non-enablement had not been established for claims 117-121. Applicants explained that the Examiner had misread the specification, and that the specification does not disclose that serum is required to cause embryonic stem cells to differentiate using the method of claims 117-121.

At page 9 of the Office Action mailed November 22, 2000, the Examiner stated that "the specification clearly indicates that serum is necessary to practice the claimed invention. As the claims are directed to a serum-free culture environment, the cells could not differentiate as required, using the claim-designated methods." Applicants respectfully disagree.

At page 44 of the present specification, in Example 6, 1% serum was used to provide attachment factors. At page 44 of the specification, lines 13-15, Applicants explained that "[i]t is expected that purified attachment factors can be substituted for the one percent serum that was used to supply such factors." Thus, serum is not required to cause embryonic stem cells to differentiate in the method of claim 117, and was not used to cause embryonic stem cells to differentiate in Example 6. Indeed, cells did not differentiate in serum-containing medium. *See* the present specification at page 44, lines 12-13 ("ES cells cultured in medium supplemented with serum (1% final concentration FBS) did not survive or form embryoid bodies").

In Example 6, serum was used to provide attachment factors. However, instead of using serum, polylysine could have been used, such that no serum would have been necessary to cause cells to attach to the culture vessel. Those of ordinary skill in the art have long used polylysine as an attachment factor. For example, *see* Freshney, R.I., "Culture of Animal Cells: A Manual of Basic Technique, Second Edition, Alan R. Liss, Inc., New York (1987) ("Freshney"), at page 140, right column, first full paragraph (attached). The Examiner has failed to provide any evidence or technical reasons why it would have required undue experimentation to facilitate attachment without using serum.

In view of Applicants' application and knowledge possessed by one of ordinary skill in the art, the claimed invention is enabled. Given Applicants' disclosure, including the examples and figures in the present application, and the knowledge possessed by those of ordinary skill in the art when Applicants' application was filed, the claimed invention could have been practiced without undue experimentation.

Conclusion

All of the stated grounds of objection and rejection have been properly traversed. Applicants therefore respectfully request that the Examiner reconsider and withdraw the outstanding rejection.

Applicant believe that a full and complete reply has been made to the outstanding Office Action and, as such, the present application is in condition for allowance. If the Examiner believes, for any reason, that personal communication will expedite prosecution of this application, the Examiner is invited to telephone the undersigned at the number provided.

Prompt and favorable consideration of this Amendment and Reply is respectfully requested.

Respectfully submitted,

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